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Direct effects of increased CO_2 concentrations in seawater on the net primary production of charophytes in a shallow, coastal, brackish-water ecosystem

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Charophytes are found in fresh and brackish waters across the globe and play key roles in coastal ecosystems. However, their response to increasing CO_2 is not well understood. The aim of the study was to detect the effects of elevated CO_2 on the physiology of charophyte species growing in the brackish Baltic Sea by measuring net primary production. Mesocosm experiments were conducted in the Kõiguste Bay (N Gulf of Riga) during the field season of 2012. Separate mesocosms were maintained at different p_{CO2} levels: ~2000, ~1000 and ~200 μatm. The experiments were carried out with three species of charophytes: *Chara aspera*, *C. tomentosa* and *C. horrida*. The short-term photosynthetic responses of charophytes to different treatments were measured by the oxygen method. The results show that elevated CO_2 levels in brackish water may enhance the photosynthetic activity of charophyte species and suggest that increasing CO_2 in the Baltic Sea could have implications for interspecific competition and community structure in a future high CO_2 world.

Introduction

Since the industrial revolution, anthropogenic emissions of carbon dioxide (CO_2) concentration has increased in the atmosphere by ~40% (Zeebe 2012). The surface of the ocean currently absorbs approximately one-third of the excess CO_2 released into the atmosphere primarily from the burning of fossil fuels, which leads to a decrease in the water pH and causes fundamental shifts in seawater carbonate chemistry (Doney *et al*. 2009). If global emissions of CO_2 continue to increase, ocean-surface pH may decrease by approximately 0.7 units by the year 2300 (Zeebe *et al*. 2008). Modelling suggests that the surface water pH in the Baltic Sea may fall up to 0.5 units by the year 2100 (Omstedt *et al*. 2012).

Research to determine the likely impacts of ocean acidification has focused primarily on describing negative effects of high CO_2 on calcifying marine organisms (both algae and animals) (Hurd *et al*. 2009, Kroeker *et al*. 2010). The response of seagrasses and marine macroalgae to ocean acidification is not well understood (Koch *et al*. 2013). Some studies have shown a negative effect of increasing CO_2 on corraline algae (Kuffner *et al*. 2008, Martin and Gattuso 2009) while studies on non-calcifying algae (e.g. Gao *et al*. 1991, Kübler *et al*. 1999, Pajusalu *et al*. 2013) and seagrasses (e.g. Palacios and Zimmerman

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2007, Hall-Spencer *et al*. 2008, Eklöf *et al*. 2012) showed a positive response to elevated CO_2.

Our understanding of the likely consequences of an increasing CO_2 concentration for macrophytes in the brackish water of the Baltic Sea is limited, and very little research has been published on this topic. Eklöf *et al*. (2012) investigated the effects of acidification on the seagrass *Zostera marina* and macroalgae from the Kattegat region of the Baltic. They found substantial effects of warming and small positive effects of acidification on the growth of seagrasses. Pajusalu *et al*. (2013) examined the response of three common Baltic Sea macroalgae species to elevated CO_2. Perennial brown algae with a slow metabolism such as *Fucus vesiculosus* did not respond to increased brackish water CO_2 concentrations while the perennial red alga *Furcellaria lumbricalis* and the fast-growing filamentous alga *Ulva intestinalis* exhibited increased net primary production.

This study focuses on charophytes, which are submerged, rooted algae with well-developed complex thalli and morphology. They grow in both fresh and brackish waters worldwide (Krause 1997, Martin *et al*. 2003). However, their response to increasing CO_2 concentrations in brackish-water ecosystems is not known. Charophyte communities are important habitats for a number of invertebrate species and epiphytic algae and they provide feeding and nursery areas for several species of fish and birds (e.g. Martin *et al*. 2003, Torn 2008). Many charophyte species develop $CaCO_3$ incrustations (Krause 1997), which are thought to be of major importance in dissolved inorganic carbon acquisition by this group (McConnaughey 1998). These incrustations have been observed to differ between different habitats; for example, in the Baltic Sea *Chara tomentosa* has much thinner $CaCO_3$ incrustations than in freshwater lakes near to the brackish Baltic Sea coast (Ray *et al*. 2003).

Most algae have carbon-concentrating mechanisms, algae can utilize HCO_3^- as a source for photosynthesis or can actively uptake CO_2 via a range of processes (Raven 1996). Based on the review of Koch *et al*. (2013), 95% of the marine macroalgal species examined possess the ability to utilise HCO_3^-. Because of the different ability of macroalgae to utilise dissolved inorganic carbon, the effect of seawater acidification varies remarkably between macroalgal species (Cornwall *et al*. 2012). Studies with the charophyte *Chara tomentosa* (Ray *et al*. 2003) and the green filamentous alga *Cladophora glomerata* (Choo *et al*. 2002) showed that algae living in the same habitat may have different strategies for carbon acquisition.

Up till now, measurements of the partial pressure of CO_2 (p_{CO2}) from the Baltic Sea are available mostly at stations located in the open waters. Measurements of p_{CO2} in the near-shore zones and areas adjacent to river mouths have until now been largely missing (Kulinski and Pempkowiak 2012). Recently some studies were initiated and data are becoming available (e.g. Saderne *et al*. 2013). Changes in the pH of the waters of Baltic Sea coastal areas are caused by high biological production and are severely affected by anthropogenic impacts such as land-use change and eutrophication (Omstedt *et al*. 2010). Algal photosynthesis (increasing pH) and respiration (lowering pH) affect the water chemistry in shallow coastal waters (Middelboe and Hansen 2007b, Semesi *et al*. 2009, Cornwall *et al*. 2013). The variability of mechanisms controlling pH along the Baltic Sea may exhibit regional differences as well as high variability on all time scales (Omstedt *et al*. 2010). Seasonal fluctuations in pH have been observed in the Baltic with maxima in May and in summer months, and minima in winter months (January–February) (Brutemark *et al*. 2011). Therefore, pH of coastal waters is more variable and difficult to predict as compared with that of the open ocean (Andersson *et al*. 2005, Saderne *et al*. 2013).

The aim of this study was to detect how carbon dioxide concentration and water pH affect the photosynthetic performance of charophytes, a group of algae that live in a highly variable pH environment in the brackish Baltic Sea. This is of importance when predicting responses of charophytes to future changes in seawater carbonate chemistry due to ocean acidification. Our hypotheses are that elevated CO_2 concentrations in brackish water increase the photosynthetic activity of charophytes and that the response varies between species.

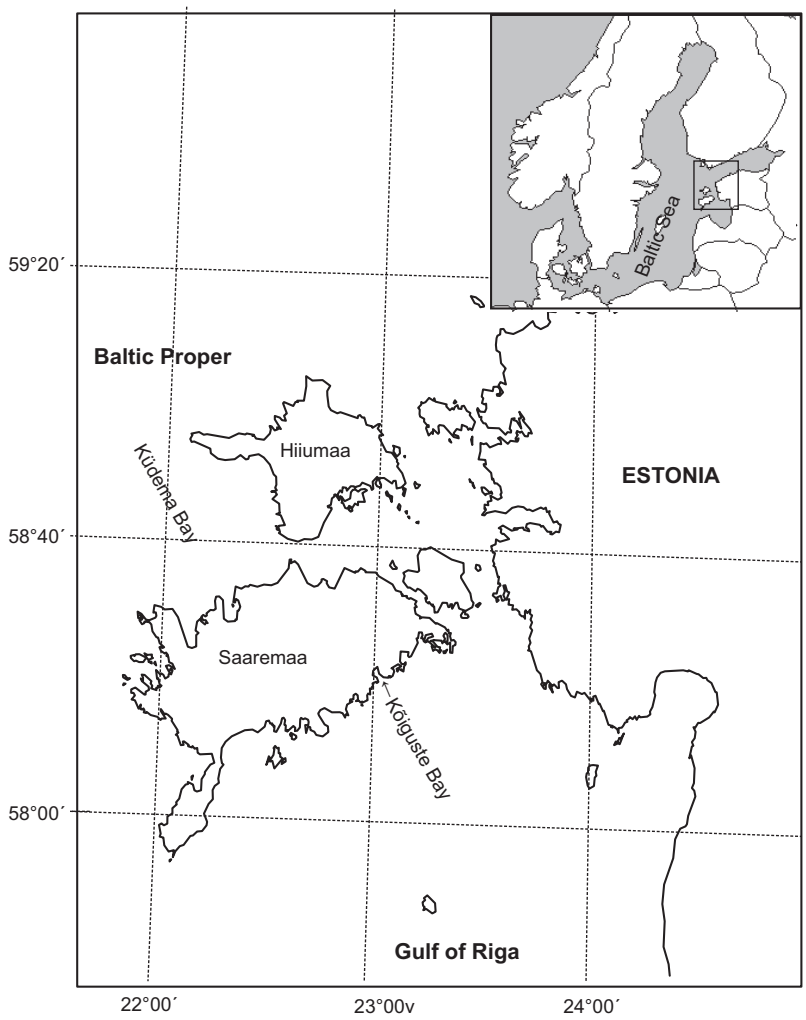

Fig. 1. Location of the study area. The arrow marks the location of the experimental site.

Material and methods

Field experiments were conducted in shallow, semi-enclosed Kõiguste Bay, northern Gulf of Riga, NE Baltic Sea (58.371°N, 22.980°E) (Fig. 1). The area is affected by a diffuse nutrient load from the moderately-eutrophicated Gulf of Riga (Astok *et al*. 1999, Kotta *et al*. 2008).

The mesocosm experiments were conducted during the field season of 2012 (experimental period: 26 June–4 July 2012). The experiments were carried out with three corticated charophyte species: *Chara aspera*, *C. horrida* and *C. tomentosa*. *Chara tomentosa* is the largest charophyte (length 30–70 cm, axis diameter 2–3 mm) in the Baltic Sea. *Chara horrida* may reach the same length, but its axis diameter remains between 1–2 mm (Migula 1897, Krause 1997). *Chara aspera* is slender and its length is often less than 10 cm. It is widespread and belongs to the most common charophytes in the Baltic Sea. Contrarily, the geographic distribution range of *C. horrida* is restricted and continuously decreasing all over the Baltic Sea, mainly due to eutrophication and coastal engineering (Blümel 2003). The species is categorised as near threatened in the HELCOM Red List (Kontula & Haldin 2013).

Charophytes were collected by SCUBA diving from the Väike Strait (58.307°N, 23.122°E) from depths down to 2.4 m. During

the sampling, the salinity of the water was ~5.9 PSU and the temperature 16.0 °C. The collected specimens were cleaned of all macroscopic epiphytes. Before the start of the net primary production measurements, the macroalgal material was subjected to 24-hour acclimatisation at the experimental site.

The charophytes were incubated free-floating in mesocosms with manipulated CO_2 concentrations for eight days. About 40 individuals of each charophyte species were placed into each mesocosm. Open plastic bags externally supported by metal frames were used as mesocosms which were placed floating in the sea and secured by anchors. Three plastic-bag mesocosms with approximate volumes of 400 l each were set up: two mesocosms with elevated p_{CO2} levels ~1000 μatm (pH ~8.05) and ~2000 μatm (pH ~7.70) and one with the natural level of ~200 μatm (pH~8.80) (control treatment). The p_{CO2} level of the control treatment was determined beforehand by measuring daily background environmental conditions. Water from the sea area adjacent to the mesocosms incubation site was sieved using a 0.25 mm mesh. Food grade pure carbon dioxide from CO_2 balloons was slowly added directly into the water in the mesocosms. A custom-made controller was used to maintain elevated CO_2 levels in the treatments. The controller switched on or off the CO_2 supply to the treatment according to the CO_2 level measured by an automatic CO_2 data logger (CONTROS™ DETECT 2.0, Germany). However, due to a response lag of the used CO_2 sensor (15–20 min), the actual CO_2 level oscillated around the lower level preset by the controller.

In parallel, the diurnal natural fluctuation of the brackish-water pH values, p_{CO2} levels and oxygen saturation were measured outside the mesocosms at 0.8 m depth in the natural shallow-water macroalgal habitat. Measurements were performed every 30 seconds during a full 24 hour cycle. The pH_{NBS} values (National Bureau of Standards scale) of each treatment were checked every day before net photosynthesis measurements with a YSI 6600V2 environmental probe (pH electrode YSI 6589FR).

The photosynthetic activity of charophytes at different p_{CO2} levels was measured every day during an eight-day period by the oxygen method. For this procedure about 0.1 g (dry weight, DW) of algal material was incubated in 600 ml glass bottles filled with water from inside the mesocosm and incubated horizontally on special transparent trays hanging outside at 0.5 m depth. Incubations with each charophyte species were performed in triplicate per treatment. Bottles without algae served as controls. The DW of the algal material was determined after drying at 60 °C for 48 h. The hourly net primary production (NPP) rates (mg O_2 g_{DW}^{-1} h^{-1}) were calculated from the differences in oxygen concentrations, measured over the incubation period (ca. 1 h) (Paalme 2005). The dissolved-oxygen concentrations were measured with an oxygen meter (Marvet Junior, MJ2000). The irradiance at the incubation depths was measured as photosynthetically active radiation (PAR) using a spherical light intensity sensor (Alec Electronics Co Ltd.). Measurements were carried out between 10:00 and 16:00.

Water samples were taken from the experimental site daily and frozen immediately for further laboratory analyses. Nutrients concentrations (total nitrogen (N_{tot}), total phosphorus (P_{tot}), phosphates ($P\text{-}PO_4$), silicates ($Si\text{-}SiO_4$) and nitrites + nitrates ($N\text{-}NO_x$) were measured in laboratory with a continuous flow automated wet chemistry analyser Skalar SAN^{plus} using the methods EVS-EN ISO 11905-1:2003, EVS-EN ISO15681-2:2005, EVS-EN ISO 16264:2004 and EVS-EN ISO 13395:1999.

The results of the field experiments were statistically analysed using the factorial ANOVA: p_{CO2} with three levels, species, experimental days and their combinations as the independent variables and NPP as the dependent variable. Bartlett's test was used to test for homoscedasticity of the data material before ANOVA. Effects were considered to be statistically significant at $p < 0.05$. When significant differences among main factors or their interactions were found, subsequent multiple comparisons of the means were done using Tukey's HSD *post-hoc* test. Statistical analysis carried out in STATISTICA 7.

Results

The fluctuation of the main environmental characteristics during the experimental period was

minimal. Salinity, water temperature and nutrient concentrations remained within the limits of typical midsummer conditions of the area. The variation in PAR was dependent on the actual weather conditions with moderate shading from clouds during most of the days (Table 1). However, NPP measurements were conducted always under saturating light conditions for charophytes.

The results of the mesocosm experiments showed that NPP rates varied both between species and p_{CO2} treatments (Table 2 and Fig. 2). The highest (absolute) NPP rates obtained during the entire experimental period were measured for *Chara tomentosa*, while *C. horrida* had higher rates than *C. aspera* (Tukey's HSD: $p < 0.05$). The charophytes *C. tomentosa* showed higher NPP rates in the water with elevated CO_2 concentrations than in the untreated water (control) (Fig. 2). The differences in the NPP rates of *C. horrida* at the p_{CO2} levels of 200 μatm and 1000 μatm were slight but at the p_{CO2} level of 2000 μatm a significantly higher photosynthetic rate was obtained than at the lower levels (Tukey's HSD: $p < 0.05$; Fig. 2). Based on a *post-hoc* test (Tukey's HSD) the elevated water CO_2 concentrations had no significant effect on the NPP rates of *C. aspera* ($p > 0.05$). Among the studied species, the photosynthetic activities of neither *C. tomentosa* nor *C. horrida* differed significantly at the control treatment and at the highest p_{CO2} level while at the intermediate p_{CO2} level the photosynthetic activity of *C. tomentosa* was significantly higher (Fig. 2). The NPP rates measured for *C. aspera* were significantly lower as compared with those of other tested charophyte species at all three p_{CO2} levels. (Tukey's HSD: $p < 0.05$; Fig. 2).

Following the dynamics of the response parameter reveals that *C. horrida* showed a remarkable immediate increase in the NPP

Table 1. Environmental conditions: salinity, values of photosynthetically active radiation (PAR) averaged during the measurement period (10:00–16:00), daily minimum and maximum water temperatures, concentrations of total nitrogen (N_{tot}), total phosphorus (P_{tot}), phosphates ($P\text{-}PO_4$), silicates ($Si\text{-}SiO_4$) and nitrites + nitrates ($N\text{-}NO_x$) in the water measured in natural conditions during the experiment.

Date in 2012	Salinity (PSU)	$PAR_{(average)}$ (µmol $m^{-2}s^{-1}$)	Water temp. (°C)		N_{tot} (µmol l^{-1})	P_{tot} (µmol l^{-1})	$P\text{-}PO_4$ (µmol l^{-1})	$Si\text{-}SiO_4$ (µmol l^{-1})	$N\text{-}NO_x$ (µmol l^{-1})
			Min	Max					
27 June	5.7	600	15.8	16.7	21.6	0.79	0.20	2.60	< 0.36
28 June	5.7	620	15.2	16.7	22.9	0.86	0.12	1.40	< 0.36
29 June	5.6	750	15.5	19.4	23.7	1.08	0.21	2.00	0.64
30 June	5.6	700	16.9	18.6	25.0	1.33	0.24	< 0.25	0.59
1 July	5.5	1060	16.9	19.5	22.7	1.13	0.12	< 0.25	0.47
2 July	5.6	1250	18.0	20.7	20.8	1.13	0.15	< 0.25	0.46
3 July	5.6	890	18.5	20.6	25.6	1.22	0.28	0.59	< 0.36
4 July	5.6	820	17.9	19.9	18.1	0.96	0.17	1.74	< 0.36

Table 2. Results of factorial ANOVA on the separate and interactive effects of CO_2, species and experimental days on the net primary production (NPP) rate in experiments. All effects are significant (*see p* values).

Source	df	MS	*F*	*p*
Intercept	1	2181.140	2415.655	< 0.005
Species	2	86.918	96.263	< 0.005
p_{CO2}	2	20.888	23.134	< 0.005
Experimental days	7	24.062	26.650	< 0.005
Species × pCO_2	4	3.705	4.104	0.005
Species*experimental days	14	12.026	13.319	< 0.005
p_{CO2} × experimental days	14	3.926	4.348	< 0.005
Species × p_{CO2} × experimental days	28	3.097	3.430	< 0.005
Error	139	0.903		

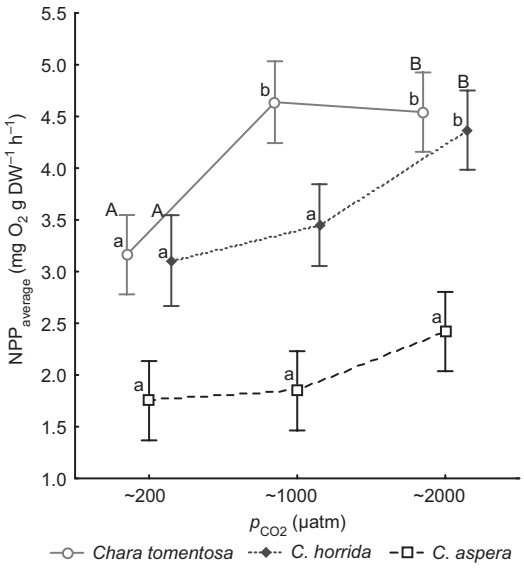

Fig. 2. Mean net primary production (NPP) rates (n = 24, error bars are ±95%CI) measured during the entire experimental period for the tested charophyte species at different brackish-water p_{CO2} levels. Within each species, same lowercase letters and between different species, uppercase letters indicate net primary production rates that are not significantly different from one another (Tukey's HSD: $p > 0.05$).

during the first days of treatment that stabilised after 3–4 days in both treatments and under control conditions. The response of *C. tomentosa* to different p_{CO2} levels was the most diverse during the first days of the experiment but the response curve stabilised by the end of incubation. At the same time the response of *C. aspera* to the treatments remained at the similar level during the whole incubation period (Fig. 3). It should be considered that photosynthetic activity measurements in the same treatment in the consecutive days cannot be treated as independent measurements, but the short time-series. Results presented in Fig. 3 illustrate the response to different p_{CO2} levels within the same species, but they also show the dynamics of how those three species adapted to the mesocosm conditions.

Factorial ANOVA (Table 2) showed that all tested factors, i.e. p_{CO2} species, experimental days and their combinations significantly affected the photosynthetic activity of three tested charophyte species.

Discussion

Our results show that elevated water CO_2 levels may enhance the photosynthetic activity of at least some charophyte species in the brackish Baltic Sea on short-term basis. The responses of the three tested charophyte species *Chara tomentosa*, *C. aspera* and *C. horrida* to elevated CO_2 concentrations in brackish water were species-specific, most probably due to differences in the ecophysiology and life strategy of the species.

In the field experiments, the highest rate of the net primary production was measured for *C. tomentosa*. The measured net primary production rates in natural conditions corresponded to those reported by Torn *et al*. (2006). The response of *C. horrida* to the elevated CO_2 levels in brackish water was less pronounced (lower absolute net primary production values) as compared with that of *C. tomentosa*, while *C. aspera* showed slight response during the experimental period. As compared with other charophyte species in Estonian coastal waters, *C. aspera* is considered the most tolerant to the variation of environmental conditions (salinity, light, bottom substrate, depth and exposure) while habitat requirements of *C. tomentosa* and *C. horrida* are more strict (Torn *et al*. 2004, Torn 2008, Torn *et al*. 2015).

Species of the genus *Chara* are primarily found in habitats with a pH range of about 6 to 9 (Moore 1986). Charophyte species respond differently to changes in water pH and, as a rule, species with a wider ecological distribution tolerate fluctuations towards lower pH levels better (Olsen 1944). For example, in our experiments the net primary production of *C. aspera* showed a significantly weaker response to elevated CO_2 with decreasing pH levels in brackish water as compared with that of the other investigated charophytes. The species-specific response of macrophytes to pH suggests that pH is one of the most important environmental factors controlling primary production in shallow coastal ecosystems. Middelboe and Hansen (2007a, 2007b) also pointed out that pH may be an important but so far overlooked factor in regulating marine shallow-water primary production.

Marine algae can have different strategies of carbon acquisition (Ray *et al*. 2003, Cornwall *et al*. 2012). Mechanisms of inorganic carbon

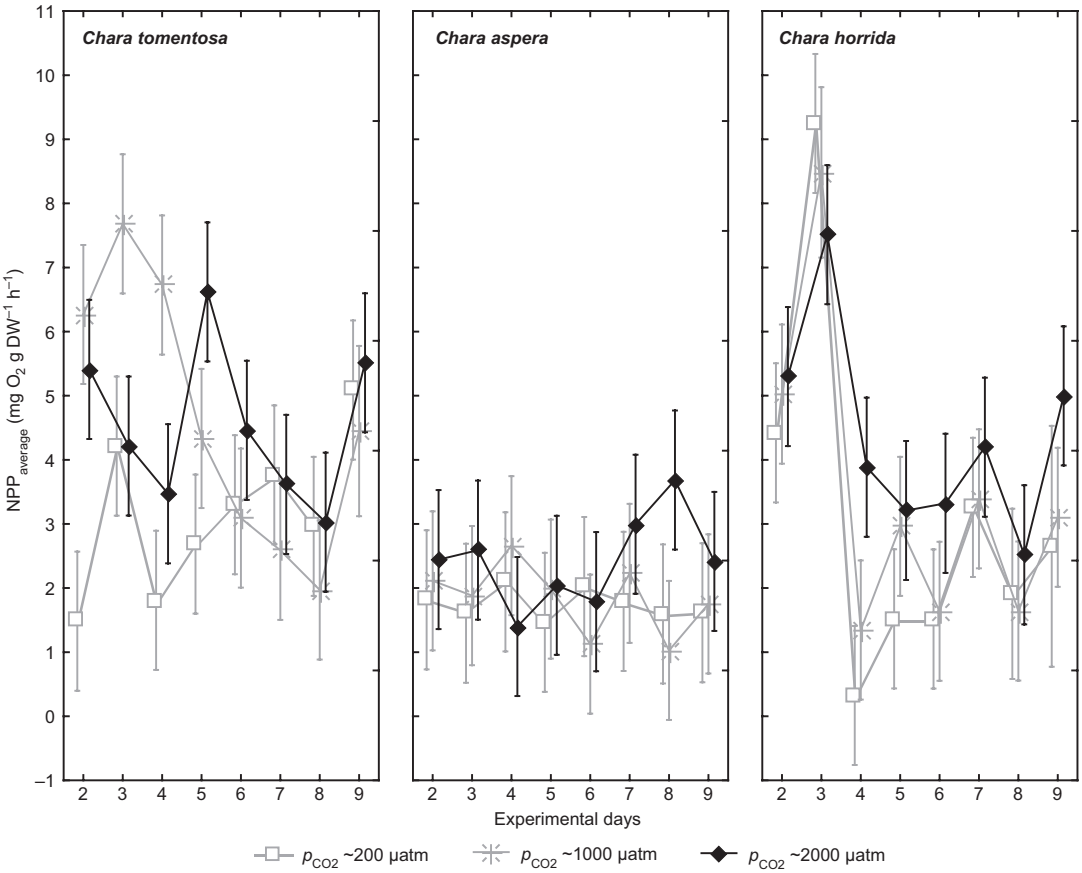

Fig. 3. The impact of p_{CO2} on net primary production (NPP) of three tested species measured in 8-days experiments with manipulated p_{CO2} concentrations (26 June–4 July 2012). Shown are means ± 95%CIs (n = 3).

acquisition can vary depending on the macroalgal species as well as the habitat (Hepburn *et al*. 2011, Koch *et al*. 2013).

With the expected increasing CO_2 content in seawater and decreasing pH, bicarbonate ions (HCO_3^-) will become slightly more available while carbonate ions (CO_3^{2-}) will be less available (Zeebe 2012). Consequently, macroalgal species that can effectively use HCO_3^- for photosynthesis may in future benefit from its increasing content in seawater (e.g. Cornwall *et al*. 2012). Many charophyte species are also able to take up HCO_3^- ions and use these as a carbon source for photosynthesis (Moore 1986). Van den Berg *et al*. (2002) indicated that the efficiency of HCO_3^- assimilation can be an important factor in the interspecific competition of submerged macrophytes. The results of their laboratory experiment demonstrated that *Potamogeton pectinatus* assimilates HCO_3^- for photosynthesis less efficiently than *Chara aspera*. It could be speculated that *C. aspera* may be a better competitor for HCO_3^- than *P. pectinatus* and will be the "winner" of this interspecific competition in a scenario of increasing CO_2 supply. Our results also suggest that increasing CO_2 in the Baltic Sea may have implications for interspecific competition in a future high CO_2 world.

The results showed that the net primary production rates varied between different brackish-water p_{CO2} treatments. At the natural level of p_{CO2}, the photosynthetic activity of charophytes was lower than at the increased p_{CO2} levels. Thus, *C. tomentosa* and *C. horrida* occurring in shallow coastal brackish water, where photosynthesis is currently limited by the natural content of CO_2 under summer conditions, may grow faster at increasing CO_2 content of brackish water in

the future. On the other hand, many algae also precipitate $CaCO_3$ incrustations and these species will not benefit from increasing CO_2 in seawater. Therefore, decreasing carbonate (HCO_3^{2-}) concentrations may cause slower calcification rates and existing calcium carbonate ($CaCO_3$) skeletons may dissolve (Anthony *et al*. 2008, Jokiel *et al*. 2008).

In freshwater, the calcification mechanism of *Chara* has been well studied (McConnaughey 1991, McConnaughey and Falk 1991). The studied ecorticate *Chara* species created separate bands of acidified and alkaline zones along their surfaces giving rise to a banding of $CaCO_3$ crystals shown as a rough texture on the thalli surface. The alkaline zones specialise in proton uptake, frequently relying on calcification to generate protons. The acidified zones specialise in proton secretion and HCO_3^- utilisation (McConnaughey 1998). It could be speculated that increased acidification in the seawater could be beneficial and appears to be supported by acidified bands. However, Kawahata *et al*. (2013) investigated *Chara globularis*, a corticate species, and did not detect alkaline and acidic areas with banding patterns in this species in fresh water. They observed that punctuated spots of $CaCO_3$ without a banding pattern outside the thallus of *C. globularis*. Ray *et al*. (2003) investigated *C. tomentosa,* a corticate species, from the brackish Baltic Sea and found perpendicular $CaCO_3$ precipitation patterns in bands. However, the brackish-water *Chara* species have much thinner $CaCO_3$ incrustations than freshwater species (Trei 1991, Ray *et al*. 2003). The mechanism and peculiarities of *Chara* calcification are not well known in the brackish Baltic Sea. In our short-term experiments, the corticated species of charophytes demonstrated tolerance to and increasing photosynthetic activity under acidic conditions.

Our earlier study (Pajusalu *et al*. 2013) on the benthic macroalgae *U. intestinalis* and *F. lumbricalis* showed that elevated CO_2 levels in brackish water may have a positive effect also on the photosynthetic activity of these species in the Baltic Sea. Moreover, the response of these macrophyte species to elevated CO_2 concentrations in brackish water also was species specific. This may lead to the conclusion that the predicted marine acidification in the Baltic Sea may cause shifts in the species composition of macrophyte communities in the future.

We established that in shallow, coastal conditions, diurnal pH and p_{CO2} were characterised by a large amplitude of natural variability. Daily fluctuations of natural brackish-water pH values from 8 to 9 are common in shallow-water macroalgal habitats in the NE Baltic in summer conditions (Pajusalu *et al*. 2013). These daily changes in pH are driven by direct effects of photosynthesis, respiration and weather conditions. While for most open-ocean biota, adapted to highly stable conditions, a slight change in pH may have significant effects, organisms adapted to highly variable conditions in the shallow, coastal waters of the Baltic Sea will most probably be less affected by such changes.

Conclusions

Increased CO_2 levels in brackish water may enhance the photosynthetic activity of charophytes in shallow coastal ecosystems under summer conditions. The effect of elevated CO_2 concentrations in brackish water on the net photosynthetic activity of charophytes was species-specific, most probably due to differences in the ecophysiology and life strategy of the species. The natural content of CO_2 in brackish water most likely limits the primary production of charophytes, especially in shallow-water macrophyte habitats. Our results suggest that increasing CO_2 in the Baltic Sea could have implications for interspecific competition and community structure in a future high CO_2 world.

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